

CLAIMS

What is claimed is:

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1. A method for isolating a phosphorylated target molecule in a sample, said method comprising the steps of:
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- a) contacting said sample with a binding solution comprising a metal chelating moiety; a salt comprising trivalent metal ions, wherein said metal ion is capable of simultaneously binding said metal chelating moiety and a phosphorylated target molecule; and, an acid;
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- b) incubating said sample and said binding solution, to form a combined mixture, for a sufficient amount of time to allow said metal chelating moiety and said metal ion to associate with said phosphorylated target molecule;
- c) separating said phosphorylated target molecules from unphosphorylated molecules by a chromatography means whereby said phosphorylated target molecule is isolated; and,
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- d) optionally determining a sequence of the isolated phosphorylated target molecule by a sequencing means.
2. The method according to Claim 1, wherein said chromatography means include a size exclusion column or a reverse phase HPLC column.
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3. The method according to Claim 2, wherein said sequencing means utilizes a mass spectrometer.
4. The method according to Claim 1, wherein said metal chelating moiety is covalently bonded to a label and said method further comprises illuminating said label with a suitable light source whereby said bound phosphorylated target molecule is detected.
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5. The method according to Claim 4, wherein said label is selected from the group consisting of a dye and a hapten.
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6. The method according to Claim 5, wherein said dye is selected from the group consisting of a benzofuran, a quinazolinone, a xanthene, an indole, a benzazole and a borapolyazaindacene.

7. The method according to Claim 6, wherein said xanthene is selected from the group consisting of fluorescein, rhodol, rosamine, rhodamine and derivatives thereof.
- 5 8. The method according to Claim 1, wherein said phosphorylated target molecule is selected from the group consisting of proteins, peptides, nucleotides, carbohydrates, phosphatase substrates, kinase substrates, lipids and inorganic phosphate.
- 10 9. The method according to Claim 1, wherein said metal chelating moiety is selected from the group consisting of BAPTA, IDA, DTPA and phenanthrolines.
10. The method according to Claim 7, wherein said binding solution has a pH about 3 to about pH 6.
- 15 11. The method according to Claim 8, wherein said metal ion is selected from the group consisting of Ga^{3+} , Fe^{3+} and Al^{3+} .
12. The method according to Claim 9, wherein said salt is gallium chloride.
- 20 13. A method for detecting and isolating phosphorylated target molecules, wherein said method comprises:
- a) contacting said sample with an activation buffer comprising gallium chloride and an acid to form an activated sample;
- b) adding a phosphate binding compound comprising a metal chelating moiety and a label to said activated sample to form a combined mixture;
- 25 c) incubating said combined mixture for a sufficient amount of time to allow said metal chelating moiety and said gallium ion to associate with said phosphorylated target molecule;
- d) adding said combined mixture to an HPLC compatible column;
- 30 e) eluting column wherein said sample is illuminated with an appropriate wavelength to detect said phosphorylated target molecule; and
- f) collecting fraction from column that contains said phosphorylated target molecule, as determined by illumination of said phosphate binding compound, whereby said phosphorylated target molecule is isolated; and
- 35 g) optionally determining a sequence of the isolated phosphorylated target molecule by a sequencing means.

14. The method according to Claim 13, wherein said HPLC column is a size exclusion column or a reverse phase column.

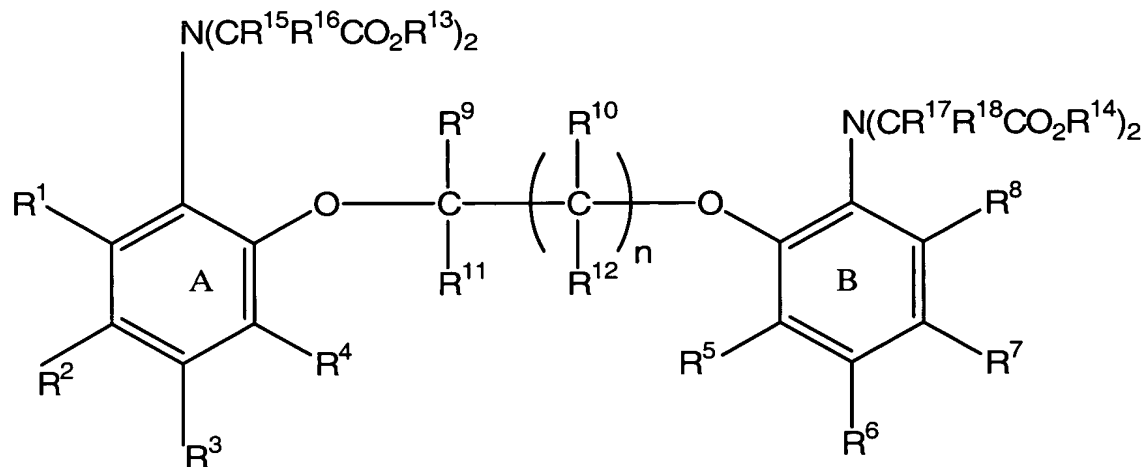
5 15. The method according to Claim 14, wherein the step of eluting includes the addition of an increasing concentration of an organic solvent.

16. The method according to Claim 15, wherein said organic solvent is methanol.

10 17. A kit for detection and isolation of a phosphorylated target molecule, wherein said kit comprises:

- a) a phosphate binding compound, comprising a metal chelating moiety that is covalently bonded to a label;
- b) an activation buffer comprising an acid and trivalent metal ions; and
- 15 c) control peptides.

18. A metal ion binding compound having the formula:



FORMULA IV

25 wherein R^1 - R^8 are independently selected from the group consisting of hydrogen, halogen, alkoxy, alkyl, aryl, amino, carboxyl, nitro, cyano, thioether, hydroxyl, sulfinyl and linker;

R⁹, R¹⁰, R¹¹ and R¹², are independently selected from the group consisting of hydrogen, linker and lower alkyl, or adjacent substituents R⁹ and R¹⁰ in combination constitute a 5-membered or 6-membered alicyclic or heterocyclic ring;

R¹⁵, R¹⁶, R¹⁷ and R¹⁸ are independently hydrogen, lower alkyl or alkyl, wherein alkyl or lower alkyl is optionally substituted by carboxyl or alkoxy;

p is 1 or 2; and,

R¹³ and R¹⁴ are independently hydrogen, -CH₂O(C=O)CH₃ or a salt;

with the proviso that at least one of R¹-R⁸ is a linker that is a single covalent bond comprising a terminal label and that at least one of the remaining R¹-R⁸ is a linker according to Formula (I) that comprises a terminal reactive group or carrier molecule provided that when the carrier molecule is dextran and the label is 2,7-difluor-3-xanthen-6-one the dextran is not attached at R³ or R⁶.

19. The metal ion-binding compound according to Claim 18, wherein said label is a xanthene.

20. The metal ion-binding compound according to Claim 19, wherein said xanthene is a fluorescein, rhodamine, rhodol, rosamine or a derivative thereof.

21. The metal ion-binding compound according to Claim 20, wherein said fluorescein is difluorofluorescein.

22. The metal ion-binding compound according to Claim 19 wherein said carrier molecule is a polymer or hapten.

23. The metal ion-binding compound according to Claim 22, wherein said polymer is a dextran and said hapten is biotin.

24. The metal ion-binding compound according to Claim 19, wherein said reactive group is an amine, a carboxylic acid, a succinimidyl ester of a carboxylic acid, a

haloacetamide, a hydrazine, an isothiocyanate, a maleimide group, an aliphatic amine, a silyl halide, a cadaverine, and an iodacetamide.

- 5 25. The metal ion-binding compound according to Claim 19, wherein said compound is selected from the group consisting of Compound 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 59, 60, and 61.